FLUORIDE ION REGENERATION OF CYCLOSARIN (GF) FROM MINIPIG TISSUE AND FLUIDS FOLLOWING WHOLE BODY GF MIOSIS LEVEL VAPOR EXPOSURE

Edgewood Chemical Biological Center
5183 Blackhawk Road, ATTN: AMSRD-ECB-RT-TT
Aberdeen Proving Ground, Maryland, USA 21010-5424

Email: edward.m.jakubowski@us.army.mil
Phone: (410) 436-8640
FAX: (410) 436-7129

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Authors

EM Jakubowski[1], JM McGuire[2], RA Evans[2], SW Hulet[1], DC Burnett[1], RA Way[1], BI Gaviola[1], JA Scotto[1], WT Muse[1], DB Miller[1], RJ Mioduszewski[1], SA Thomson[1].

[1]Research and Technology Directorate, US Army ECBC, APG-EA, Edgewood, MD 21010, USA;

[2]Geo-Centers, APG-EA, Edgewood, MD 21010, USA.

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Introduction

Chemical warfare nerve agent biomarker methods:

- Cholinesterase activity,
- Nerve agent hydrolysis products,
- Fluoride ion regenerated alkyl methylphosphonofluoridate (G-agents)
  - human plasma,
  - red blood cells,
  - tissue

New analytical method:

- ammonia CI,
- a large volume injector (LVI) with Tenax® insert, and
- stable isotope ($^{2}H_{11}$-GF) internal standard.

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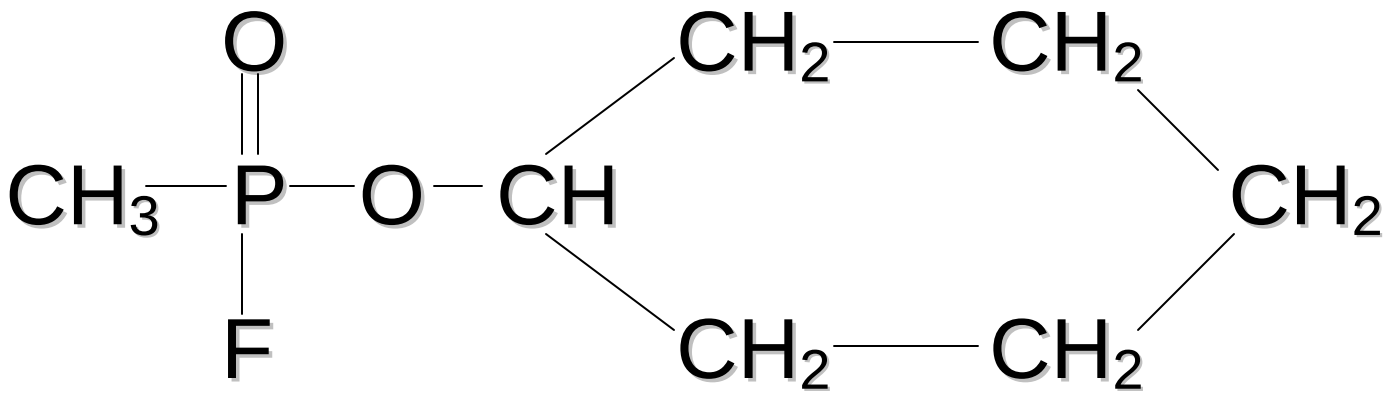
Cyclosarin (GF)



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Objectives

- Develop method for GF exposed samples using GC-MS with positive ammonia chemical ionization and stable isotope standards.
- Explore performance characteristics including:
  - Column selection
  - optimizing LVI and oven parameters,
  - reportable range,
  - accuracy and precision,
  - detection limits.
- Spike animal whole blood/tissue at a series of GF levels
  - Analyze Recovery Data
- Analyze minipig samples after whole body GF exposure

Experimental Methods: Materials

- GF **:** CASARM grade prepared and analyzed at ECBC and diluted with hexane or ethyl acetate.
- Deuterated GF ($^{2}H_{11}$): Synthesized at ECBC isotopic purity >99.9% by mass spectrometry
- C_{18} SPE cartridges: 200mg (Waters Associates, Millipore Corp., Milford, MA)
- Acetate buffer (pH=3.5)
- Potassium fluoride: 6 M
- Chemicals**:** All other chemicals were procured commercially at ACS reagent grade or higher.

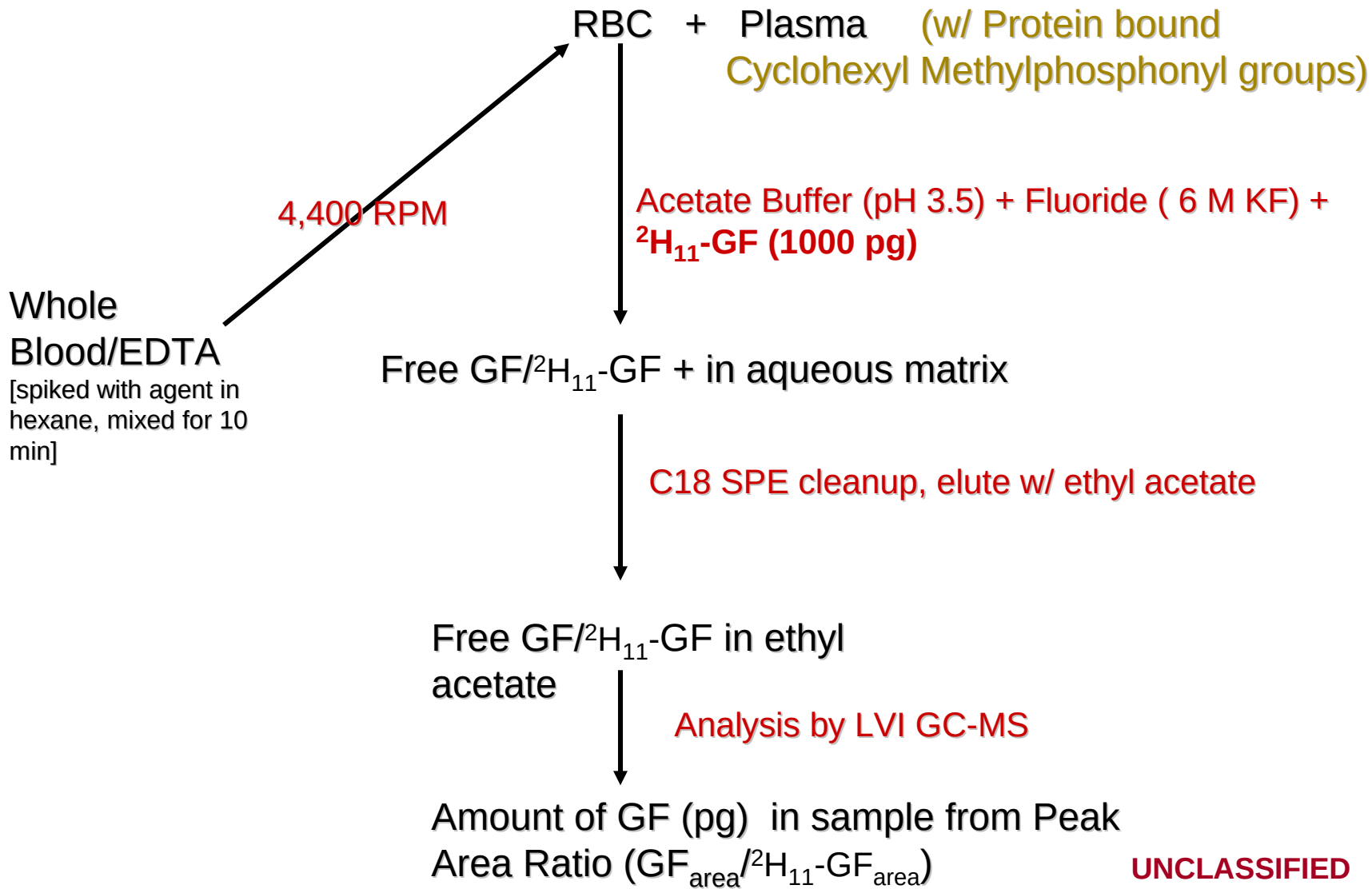
Minipig Blood Sample Collection

Inhalation Exposure Samples:

Whole blood from GF exposed minipigs was collected (with EDTA) via external jugular catheter allowing serial blood sample collection before, during, and after inhalation exposure.

Samples were centrifuged at 4400 rpm for 5 min.The resulting red blood cell pack and serum/plasma samples were analyzed for regenerated agent by the addition of acetate buffer and fluoride ion.

Sample Process Flowchart



Experimental Methods: Analysis

- **GC** Model 6890 (Agilent Technologies, Wilmington, DE)
- **LVI Sample Introduction:** Agilent PTV: Inject 50 uL of extract, initial temp 0°C, initial time 5.1 min, final temp 260°C, rate 720°C/min, vent time 5.00 min, vent flow 300 mL/min, purge flow 50 mL/min, purge time 7.85 min.
- **GC Column:**
  - 14%-Cyanopropylphenyl 86%-Dimethylpolysiloxane: 30 m x 0.32 mm x 1 μm film(Rtx-1701, Restek Inc., Bellefonte, PA)
- **GC Oven program:** Carrier He @ 3 mL/min (63 cm/sec),Temp Program: - 35°C(5.3 min) to 164°C @ 50°C/min to 178°C @ 2°C/min to 270°C(3 min) @ 50°C/min.
- **Detection:** MSD (Model 5973 MSD, Agilent Technologies, Wilmington, DE) SIM mode, ammonia CI, Source & Quad Temp 150°C
  - GF ions: Target-198.1 ($[M+18]^+$) & Qualifier 215.2 ($[M+35]^+$), Retention time 10.61 min
  - Internal standard ($^2H_{11}$-GF):Target-209.1 ($[M+18]^+$) & Qualifier 226.2 ($[M+35]^+$), Retention time 10.53 min

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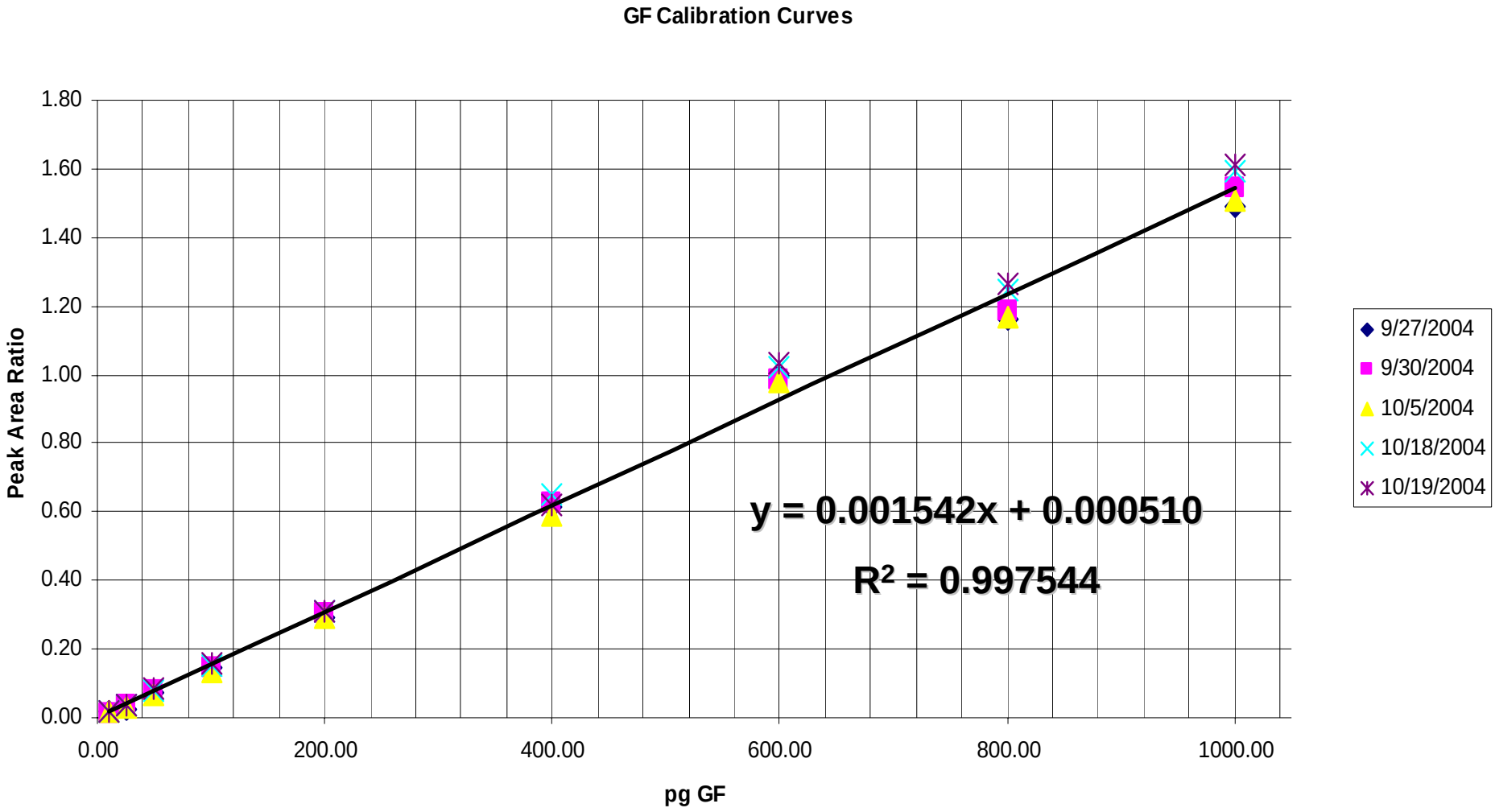
Results and Discussion

- Positive ion NH_3 chemical ionization (CI) was chosen over CI using negative ion NH_3, positive/negative ion CH_4, or positive/negative ion isobutane.
  - NH_3 afforded better sensitivity then CH_4 or isobutane
  - Positive ion mode less sensitive to source conditions
- Ratio of $[M+18]^+/[M+35]^+$ was used as tuning benchmark to set NH_3 pressure
- LVI flow rates and injector port purge times are critical parameters to optimize for each solvent and target analyte.
- Tenax packed injector port important.
- Detection limit was in the low picomole range.
- Calibration Curve Range 10-1000 pg on column (50 uL injection)
- Figure 1 is a overlay of five calibration curves from over a three week period.

Figure 1. Calibration Curves



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Results and Discussion

- Peak-to-peak signal/noise at the lowest standard (10 pg) was over 100.
- Method can be used with blood or tissue samples.
- Figure 2 is a representative plot of lung tissue spiked with GF.
- Figure 3 is a representative plot of the regenerated GF (R-GF) from a minipig eye sample after whole body exposure to GF at 0.16 mg/m^3 for 10 min.
- Figure 4. minipig RBC Sample at 1500 min post exposure (0.16 mg/m^3 for 10 min).
- Figures 5-7 compare GF and GB uptake in the minipig.
- Figures 8 & 9 demonstrate late occurring peaks in R-GF profiles.
- Figures 10 compares R-GF and R-GB from tissue samples.
- Figure 11 presents the ratio of R-GF of Eye/RBC versus Ct
- Figure 12 shows that trends continue with near lethal exposures.

Figure 2. GF lung tissue spike (100 pg)

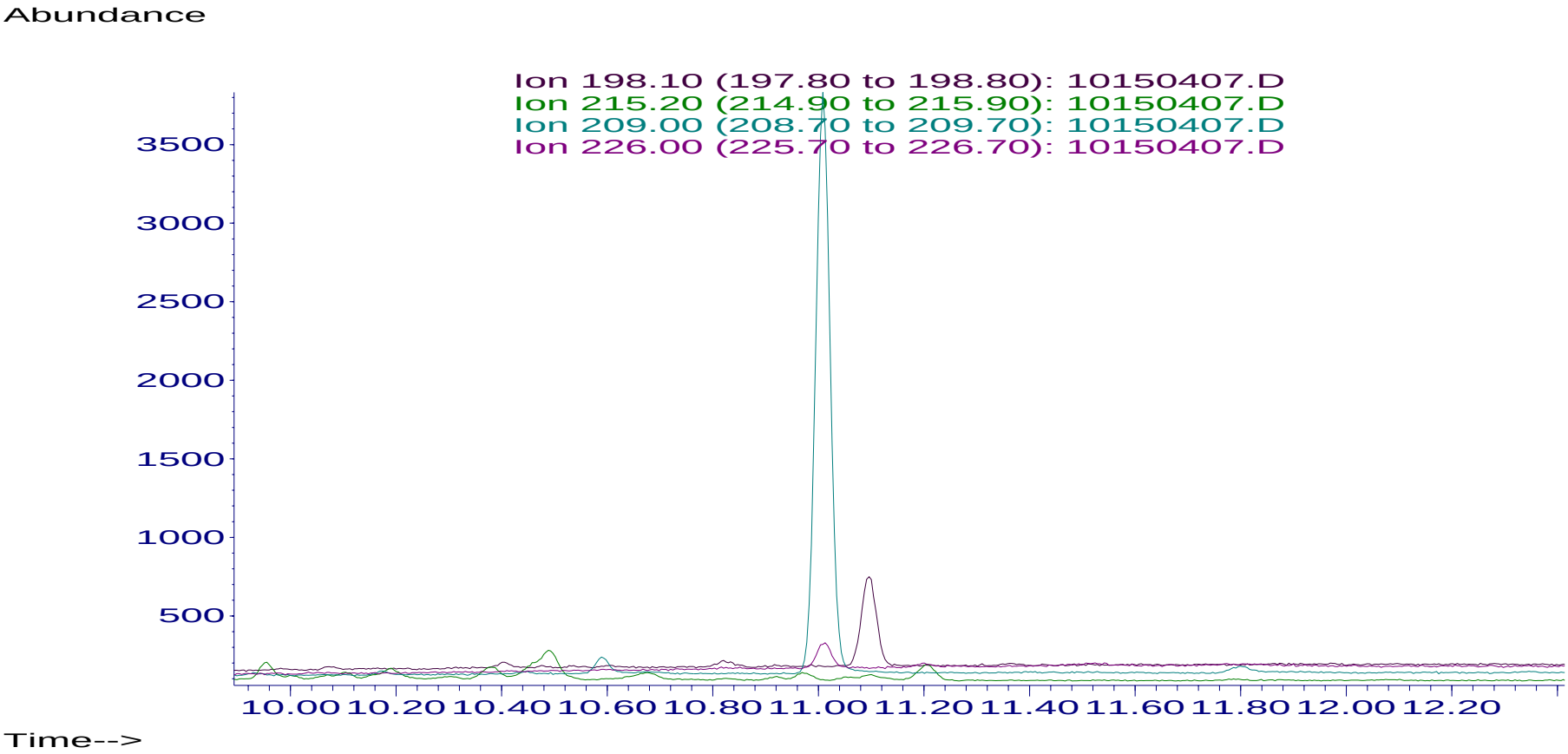


Figure 3. Minipig Eye Tissue Sample Post Exposure (375 pg on column)

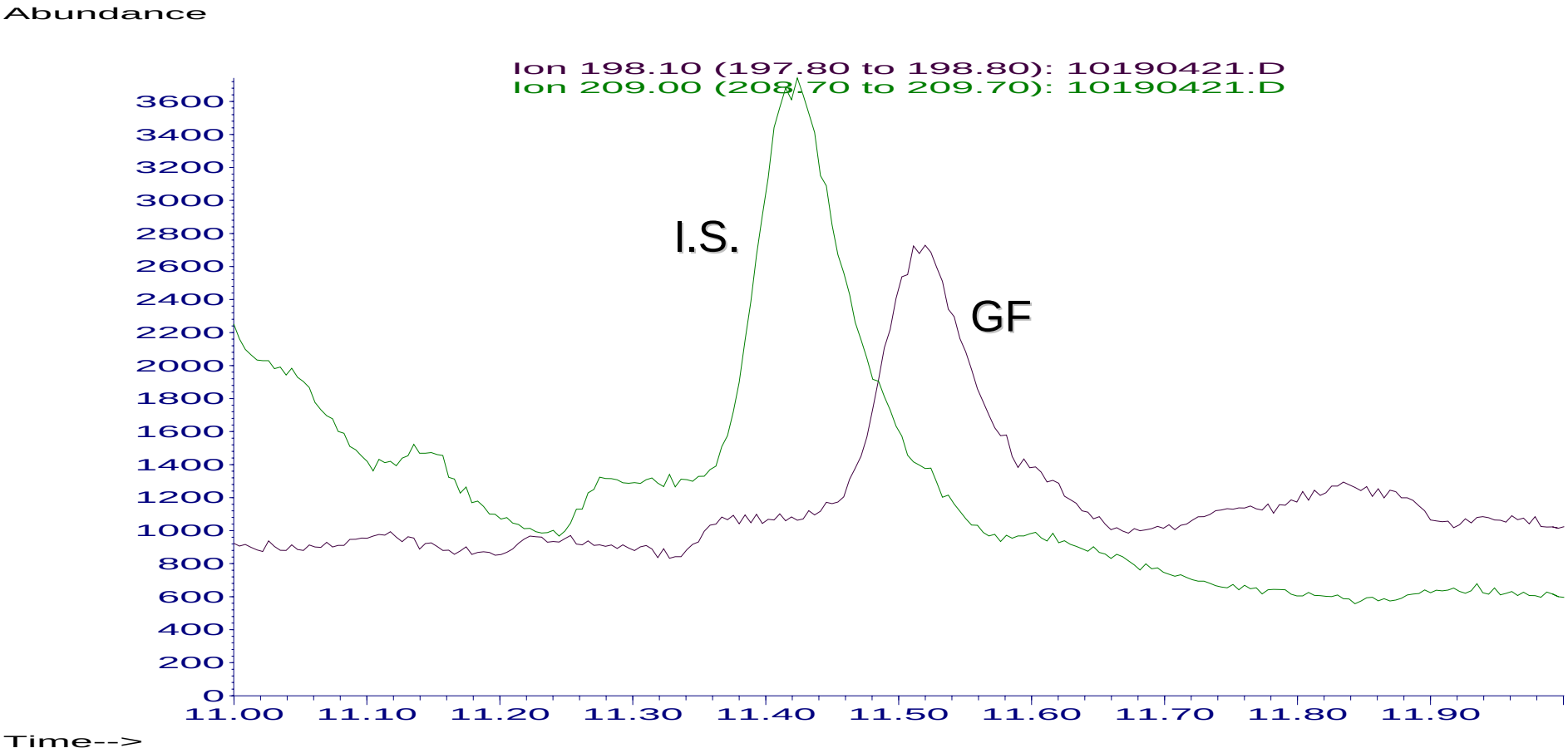


Figure 4. Minipig RBC Sample at 1500 min

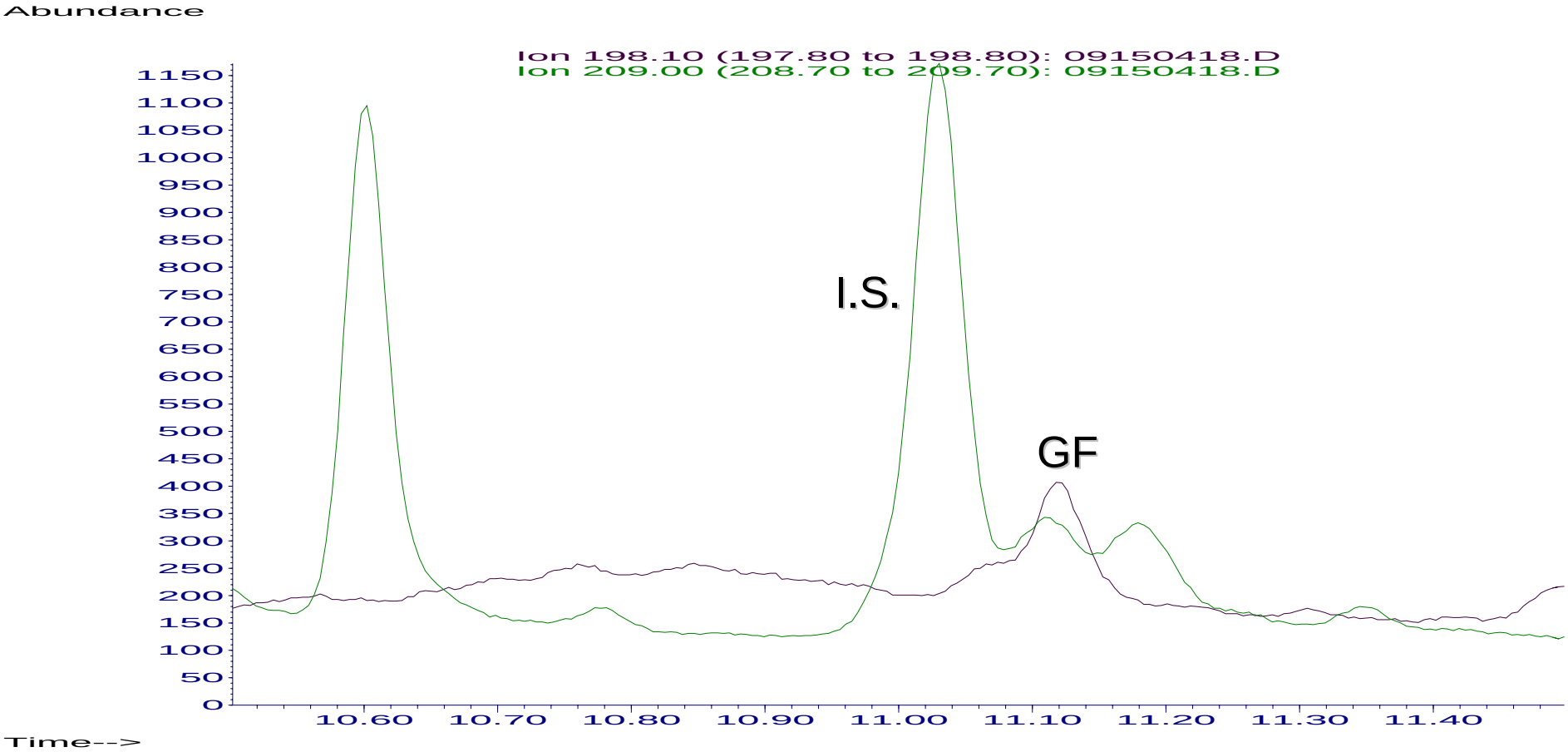


Figure 5. GF vs GB Uptake in RBCs (ng/g)

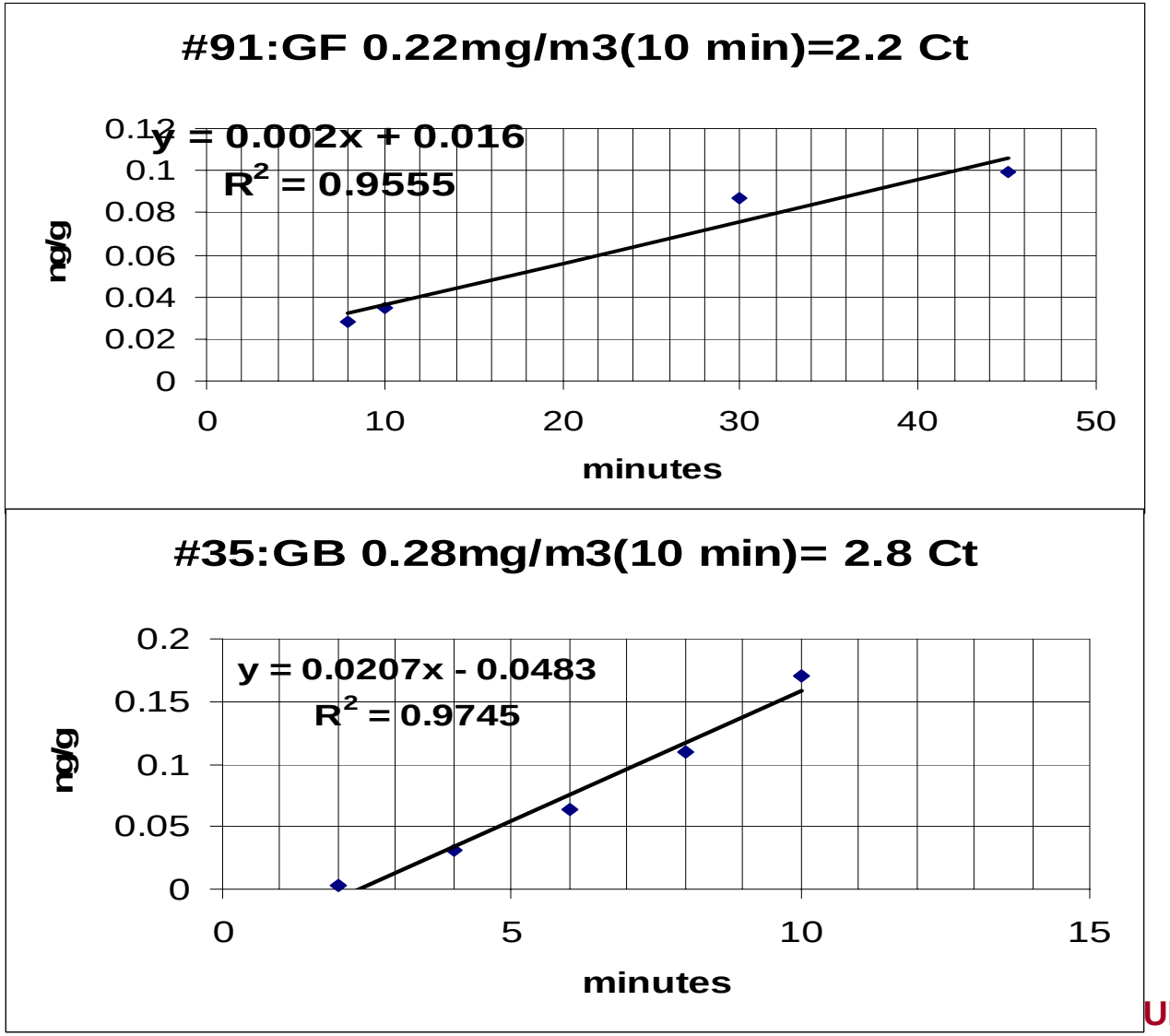
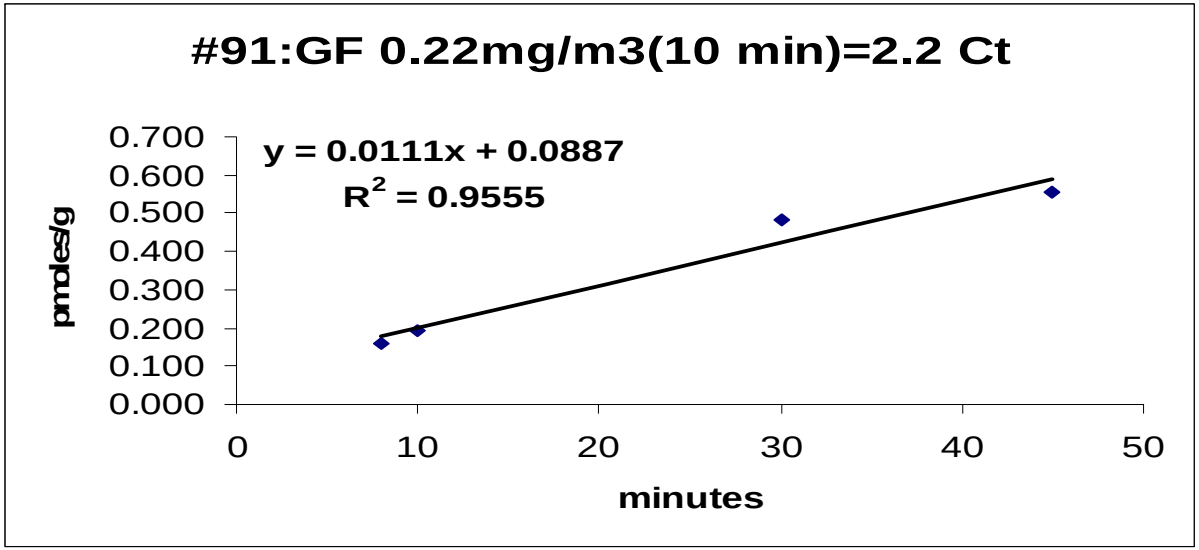
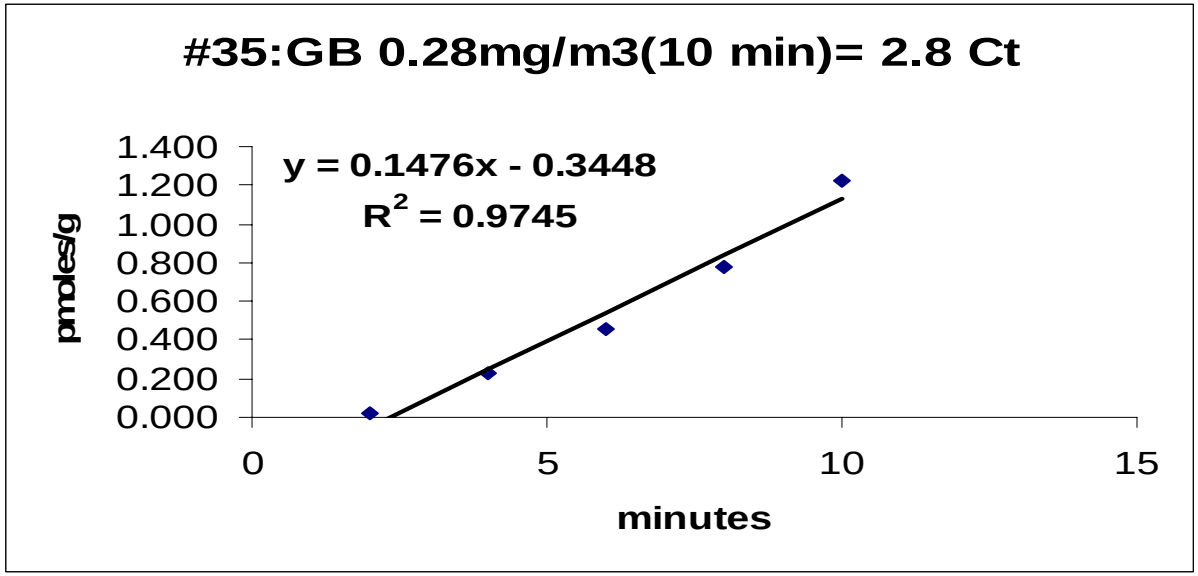


Figure 6. GF vs GB Uptake in RBCs (pmoles/g)

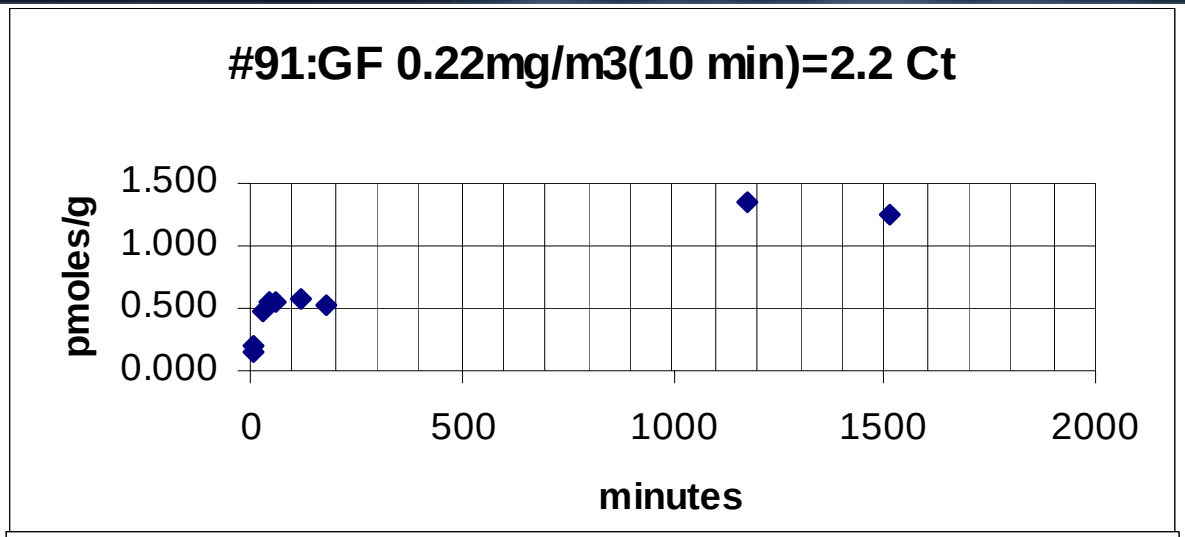


0.0122 mmoles(GF)/m³*t

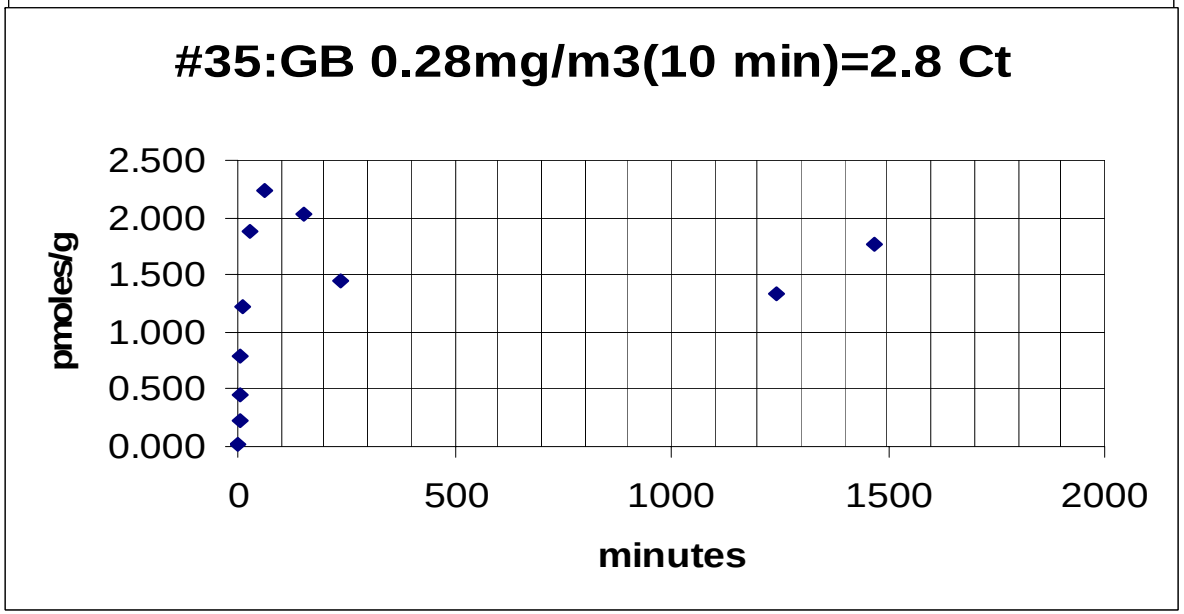


0.0200 mmoles(GB)/m³*t

Figure 7. R-GF vs R-GB from RBC, 24 Hr +



0.0122 mmoles(GF)/m³*t



0.0200 mmoles(GB)/m³*t

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Figure 8. R-GF from Exposed Minipig

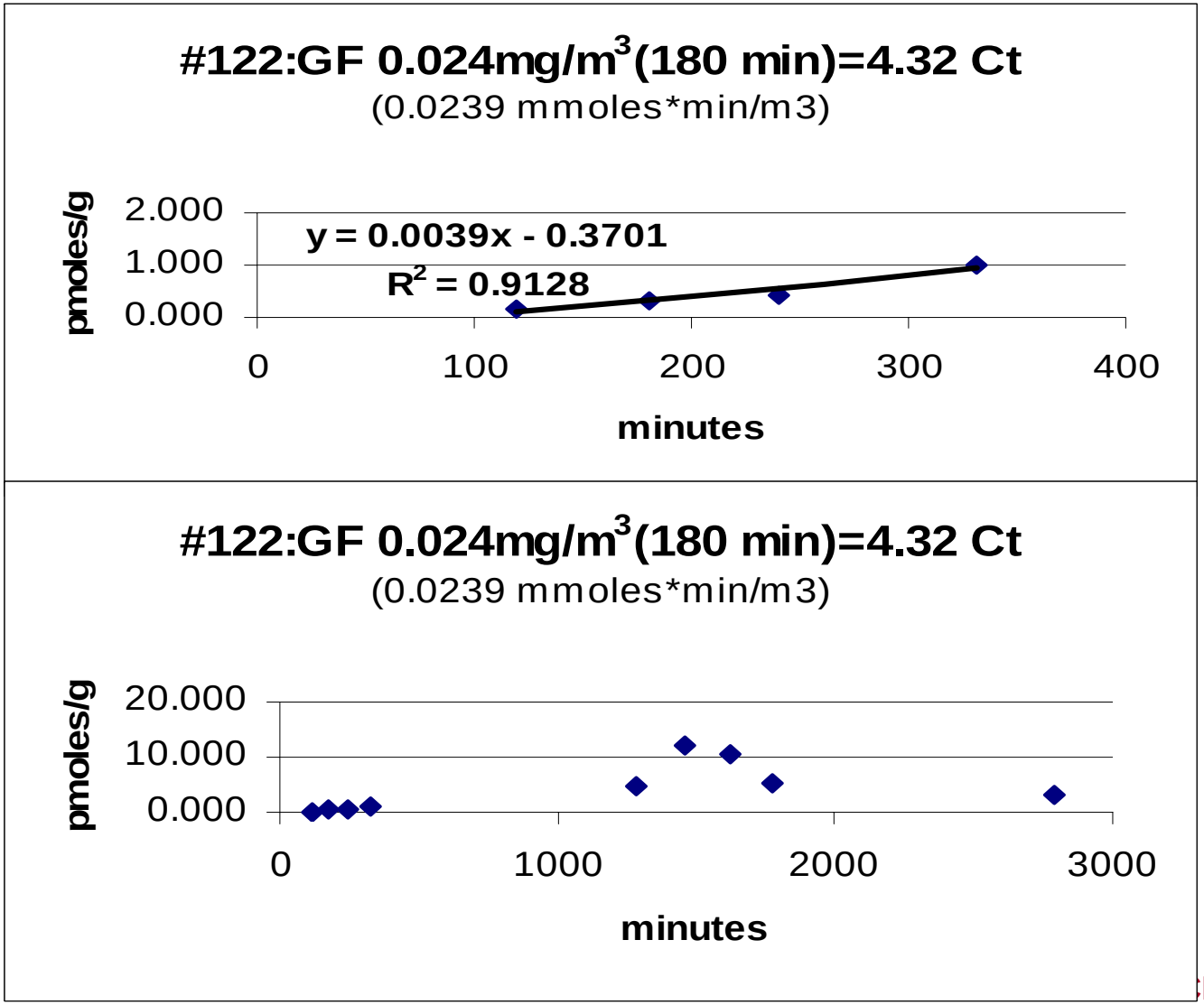

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Figure 9. R-GF Recovery Over Time

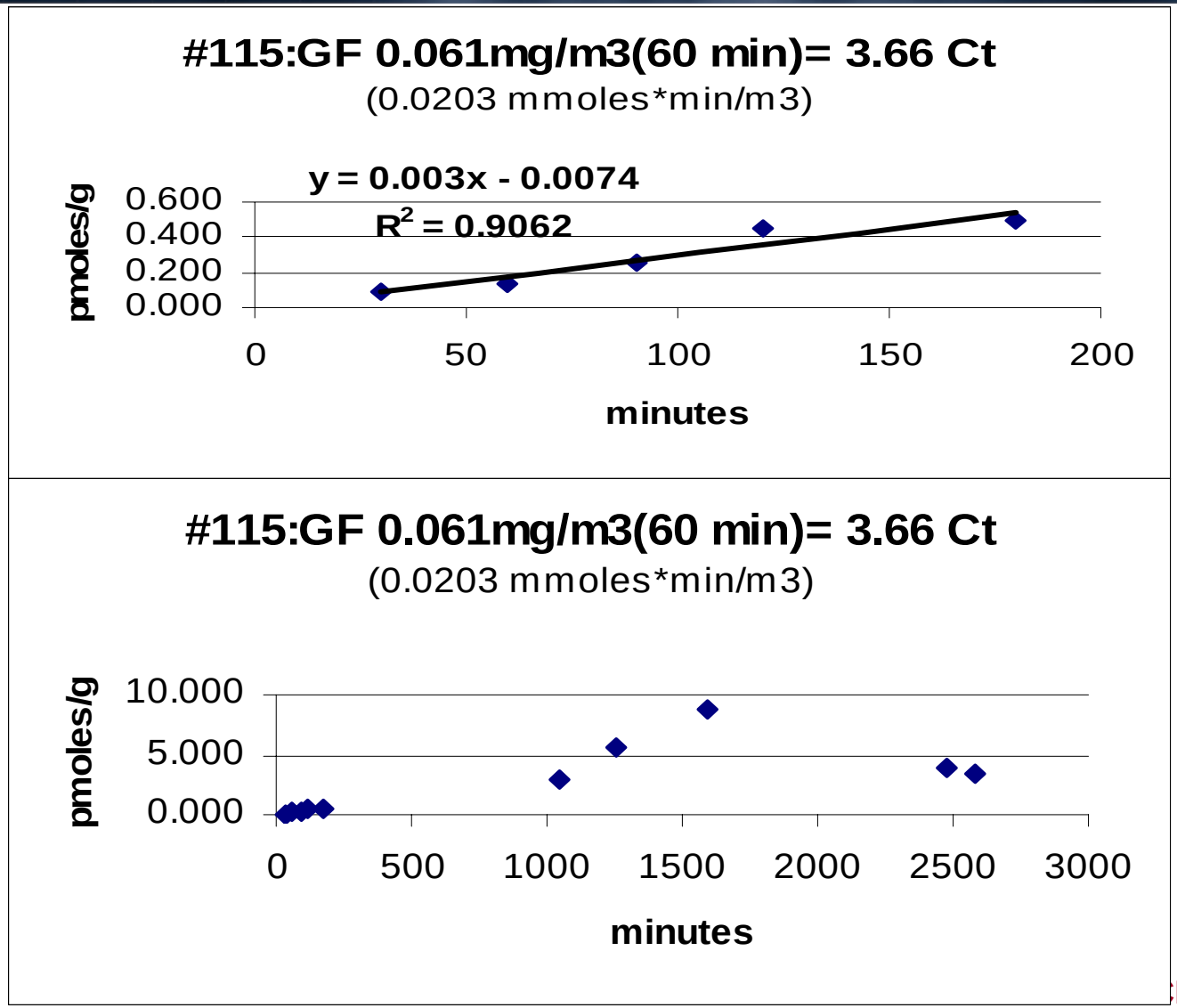


Figure 10. R-GF from Tissue Samples

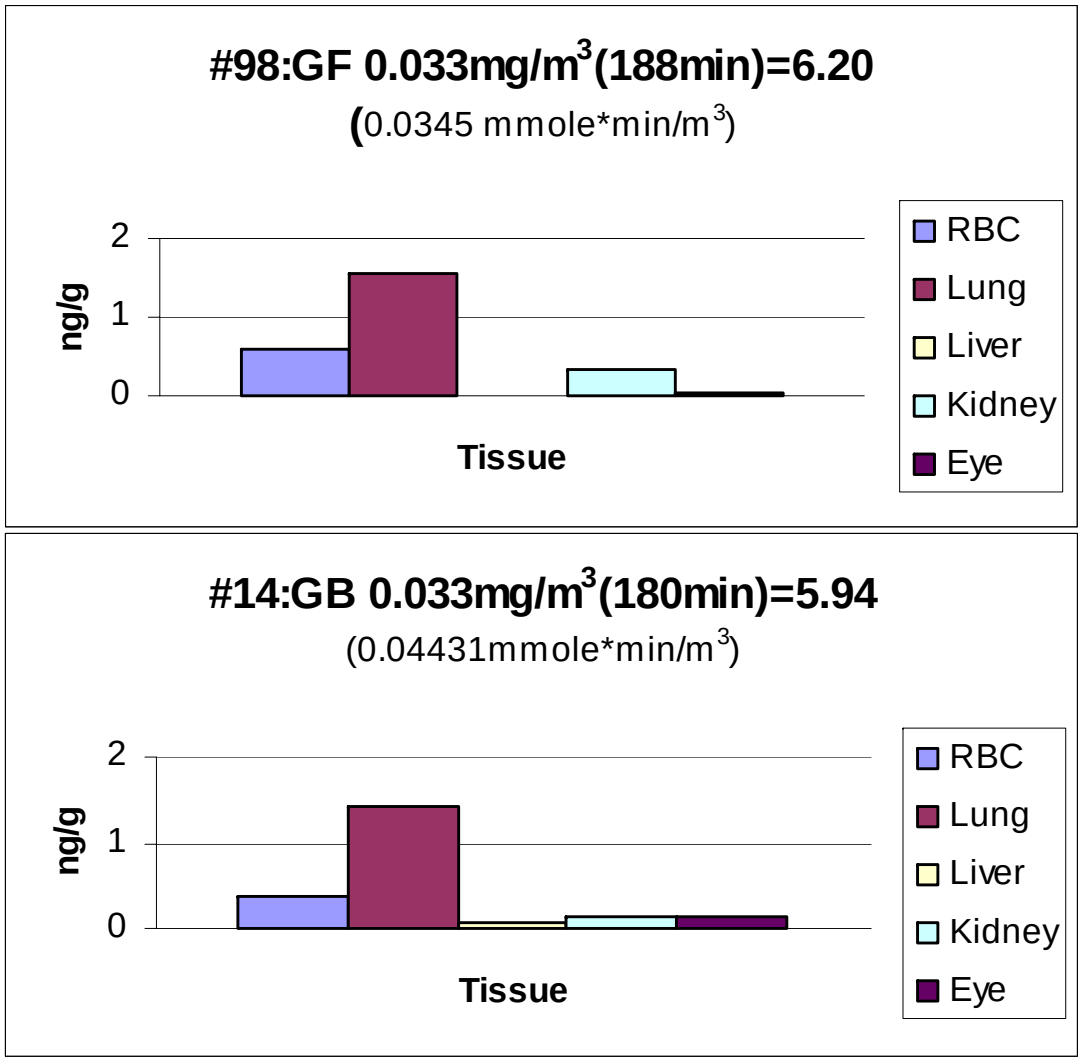


Figure 11. R-GF in Eye Tissue Relative to RBC

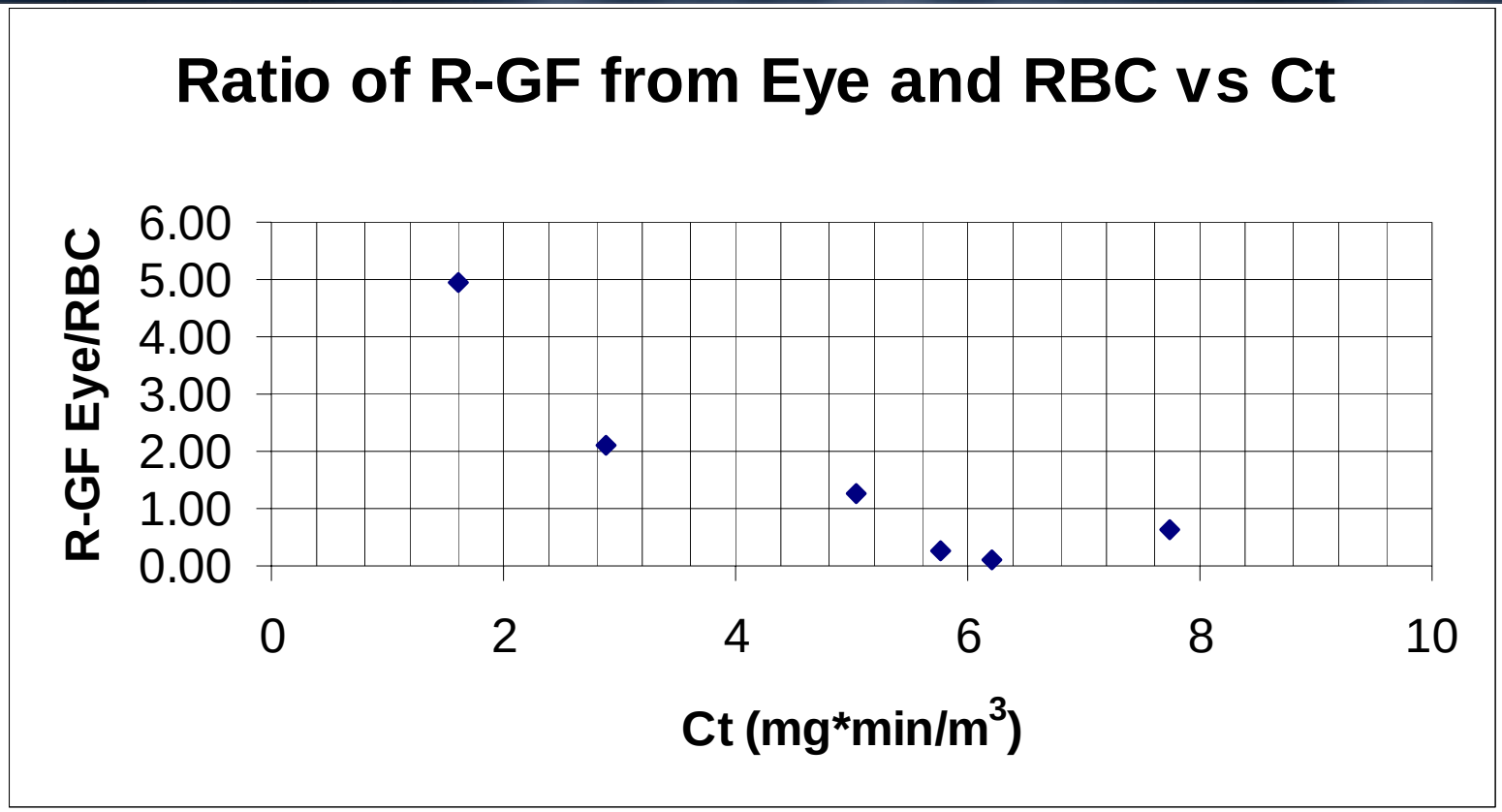
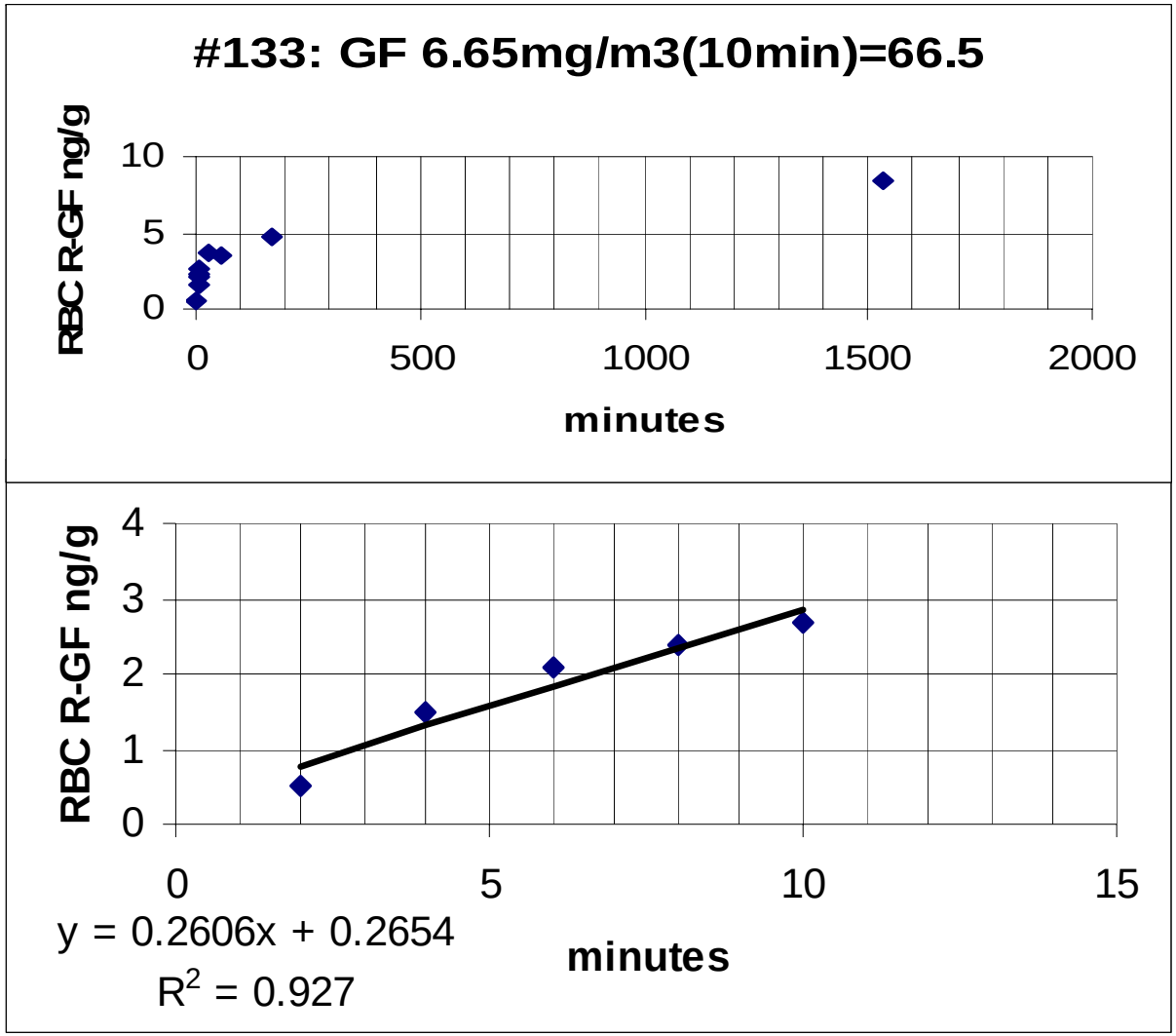


Figure 12. R-GF at Near Lethal Exposure



Summary/Conclusions

- The isotope dilution LVI method is sensitive enough to quantify R-GF in blood and tissue after miosis level exposure
- Results indicated the ability to quantify GF down to ~400 pg/mL of extract despite the complexity of the matrix.
- Conditions that needed to be optimize for the LVI included injection volume, initial temperature, initial time, pressure, liner packing, and flow rate.
- The uptake of GF as reflected by R-GF levels appears to be much slower than GB.
- Maximum recovery of R-GF was ~24 hours after the beginning of the exposure for all levels of exposure to date. R-GB maximum is reached soon after exposure stops.